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Effect of Nicotine on Cholesterol-Induced Atherosclerosis in the Rabbit

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Nicotine was administered orally in three dosage levels to female rabbits fed a 1 per cent cholesterol, 5 per cent oil diet for 24 weeks. Serum cholesterol and phospholipid values and electrocardiograms, both with and without ergonovine stress, were obtained at eight week intervals. Gross and microaortic pathology and cardiac micropathology were determined at the end of the experiment. Serum cholesterol and phospholipid values and aortic atherosclerosis were not affected by the addition of nicotine. Mortality, the incidence of positive ergonovine stress tests, myocardial necrosis and fatty metamorphosis, and peripheral vascular changes were increased. A dose-response relationship could not be established for nicotine.

IT HAS been reported[1, 2] that nicotine increases the plasma cholesterol levels of male rabbits on a cholesterol-fortified diet. The purpose of this study was to determine the effect of graded doses of nicotine plus a high cholesterol-oil diet on atherosclerosis using the following criteria: serum cholesterol and phospholipid levels, electrocardiographic changes both before and after ergonovine stress, and gross and micropathology of the heart and contiguous vessels.

METHODS

Six groups of 12 albino, New Zealand, six-week old, female rabbits per group were established. All animals weighed between 1.7 and 2.1 Kg. Group 1 received the stock diet of Purina rabbit chow and water ad libitum. For group 2 the food was impregnated with 1 per cent cholesterol and 5 per cent cottonseed oil. Group 3 was fed the stock diet plus the human nicotine equivalent by body weight of two packs of cigarettes daily in the drinking water. This dose is based upon the report that 4 μg. of nicotine per os produces the same psychic effects as one cigarette in the chronic smoker.[3] Using 70 Kg. as the average adult human weight, the daily two pack equivalent is 2.28 mg. nicotine/Kg. body weight, the one-half pack equivalent is 0.57 mg./Kg., and the one-eighth pack equivalent is 0.142 mg./Kg. Groups 4, 5, and 6 received the group 2 diet and one-eighth, one-half, and two pack equivalents of nicotine respectively. In order to reduce the possibility of acute effects from the nicotine, the dose was gradually increased in order to produce tolerance. Each group was started with one-twelfth the calculated daily dose for the first three days. This amount was increased by a similar quantity every three days until the full dose was being administered on the thirty-sixth day.

Determinations of body weight, serum cholesterol, and phospholipid and electrocardiographic activity both with and without ergonovine were made initially and every eight weeks thereafter for a period of 24 weeks.

Electrocardiography was performed with an Edin C. C.-D. C. amplifier and a Brush Model BL-201 oscillograph at a paper speed of 25 mm./second. The usual limb leads and a chest lead positioned over the heart were used. Electrodes were prepared from silvered hypodermic needles. The rabbits were unanesthetized but were immobilized by use of a rabbit board through which the head could be lowered below the plane of the body. Records were obtained prior to and at 1, 3, 5, and 10 minutes after the injection of 0.05 mg./Kg. ergonovine maleate into the marginal ear vein.

At 24 weeks all surviving animals were killed and in addition to examination for microscopic pathology the degree of aortic sclerosis was grossly graded on a modified 0 to 4 scale. Grade 0 was considered to be less than 2 per cent of the total surface involved, grade 1, up to 10 per cent,

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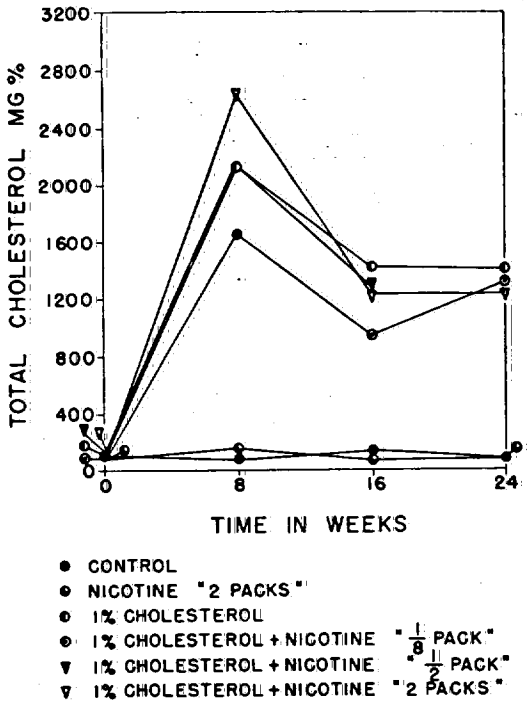

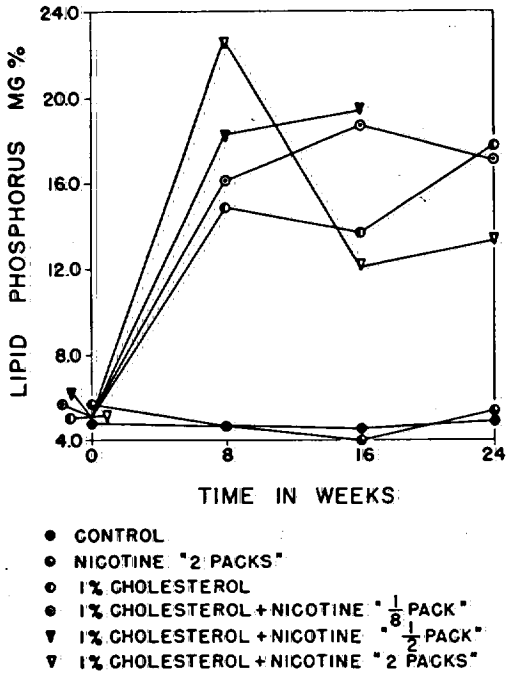

FIG. 1 *Left.* Average serum cholesterol values obtained at eight week intervals.
FIG. 2 *Right.* Average phospholipid values obtained at eight week intervals.

grade 2, up to 20 per cent, grade 3, up to 40 per cent, and grade 4, up to 80 per cent. When significant thickening of the lesions were observed, 0.5 was added to the grade. All values were equated to a maximal value of 4 by direct proportion. For the purpose of grading, the aorta was arbitrarily divided into arch, ascending, and thoracic aortic areas. The areas involved were independently estimated by two observers. In borderline cases the dimensions of the plaques were determined by use of a comparator with appropriate reticles.

The heart and aorta were fixed in 10 per cent formalin. Frozen sections were made through the arch of the aorta and the base and apex of the heart. Tissues were stained with oil red O and hematoxylin counterstain.

RESULTS

Serum Cholesterol-Lipid Phosphorus. Figures 1 and 2 illustrate the changes in the serum cholesterol and lipid phosphorus levels. Although an initial linear dose relationship occurs for the nicotine and the serum cholesterol levels, this did not continue, nor was it significant due to large individual variations. Values for the one-half pack group are not given after the sixteenth week because of the high mortality in this group between 16 and 24 weeks. The 3 nicotine-cholesterol groups showed significantly greater mortalities (70, 91, and 50 per cent) than any of the other groups at 24 weeks (control 8 per cent, nicotine 0, cholesterol 8 per cent). Although there were indications of circulatory failure in those animals who died, correlation of the deaths with cardiac involvement was not attempted.

Ergonovine Stress Test. The electrocardiograms of the cholesterol and nicotine-cholesterol groups did not begin to demonstrate increased abnormalities related to the administration of ergonovine until the sixteenth week. The results as given in table 1 are obtained from the 24 week records. The 16 week records were used for those animals that died between the 16 and 24 week periods. Two criteria of ergonovine stress were selected: A was an ergonovine-induced S-T depression of 1.0 mm. (2 cm. - 1 mv) below the isoelectric level and/or a flattening or inversion of the T wave in leads II, III, or IV. Criterion B was the same as that of A except that flattening of the T wave was eliminated as a posi-

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TABLE 1.—*Percentage of Rabbits Exhibiting Positive Ergonovine Stress Test at 24 Weeks*

Group no.	Diet	Criterion A* (%)	Criterion B† (%)
1	Control	27.3 (3 of 11)	18.2 (2 of 11)
2	Nicotine	25.0 (3 of 12)	0.0 (0 of 12)
3	Cholesterol	58.3 (7 of 12)	33.3 (4 of 12)
4	Cholesterol plus nicotine "1/8"	71.4 (5 of 7)	71.4 (5 of 7)
5	Cholesterol plus nicotine "1/2"	71.4 (5 of 7)	57.2 (4 of 7)
6	Cholesterol plus nicotine "2"	72.5 (8 of 11)	54.6 (6 of 11)

*Drug-induced depression or elevation of the S-T segment of 1 mm. or more below the isoelectric level and/or "flattening" or inversion of the T wave in lead II, III or IV.

†Same as A with the elimination of T wave "flattening."

tive sign of coronary insufficiency. It was felt that criterion B was more valid, as a number of animals in all groups demonstrated the flattened T wave prior to ergonovine stress. The effect of both pentobarbital anesthesia and the position of the animal on the positivity of the electrocardiogram was determined in a number of cases. It was observed that pentobarbitalization had no effect on positivity, whereas the position, that is, on the back as used in the present study or the side as used by Rinzler,[4] markedly affected the results. Animals that were negative in the back position were often positive on the side and vice versa. It may be seen from table 1 that a consistently higher percentage of nicotine-cholesterol animals (groups 4 to 6) demonstrated positive electrocardiograms in response to ergonovine than did the cholesterol only group (group 3). Figure 3 illustrates electrocardiograms obtained from a nicotine-cholesterol animal. The typical S-T depression can be seen in the record of the nicotine-cholesterol animal.

Aortic Pathology. Results of the gross grading of the aortas are given in table 2. Rabbits in all groups fed cholesterol exhibited marked involvement of the aortas although there was no difference between the cholesterol

TABLE 2.—*Summary of Gross Grading of Rabbit Aortas for Atherosclerosis*

Group no.	Diet	Sample size	Ascending aorta	Aortic arch	Thoracic aorta	Mean
1	Control	12	0	.35	.62	.32
2	Nicotine	12	0	0	0	0
3	Cholesterol	12	3.59	4.00	2.33	3.30
4	Cholesterol plus nicotine "1/8"	6	3.47	4.00	1.40	2.95
5	Cholesterol plus nicotine "1/2"	7	3.66	3.92	2.15	3.24
6	Cholesterol plus nicotine "2"	10	3.91	3.95	2.13	3.33

(group 2) and any of the nicotine-cholesterol groups (4 to 6).

Cardiac Micropathology. In the control animals (group 1) there were small amounts of adipose tissue around the coronary vessels. No apparent abnormalities were observed in the walls of any of the blood vessels.

The nicotine group (2) demonstrated minimal paravascular depositions of adipose tissue as well as minimal fatty metamorphosis of myocardial cells. There was, however, in all animals of this group considerable thickening and fibrosis of the small branches of the coronaries.

The cholesterol only group (3) exhibited somewhat varying changes. In general, the coronaries were thickened with advanced atherosclerotic changes. These changes appeared to be most pronounced in the subendocardial vessels. In some arteries closely adjacent to the endocardium, the lumen was reduced in size to approximately one tenth the diameter of the vessel. In 1 animal small areas of necrosis were observed, while 3 animals demonstrated fatty metamorphosis of the myocardial fibers.

The nicotine plus cholesterol groups (4 to 6) exhibited atherosclerotic changes essentially similar to those of the cholesterol only group (3), with certain notable additions. These additions were greater amounts of fatty metamorphosis, and the presence of actual early necrosis of the myocardial tissues in all

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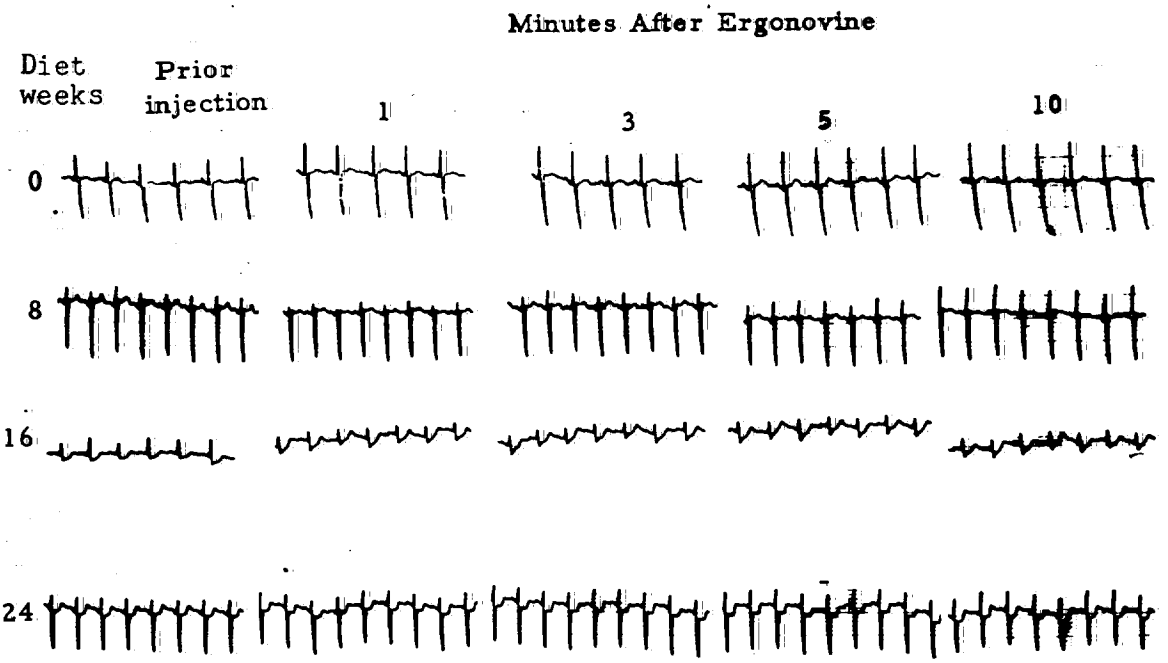

FIG. 3. Serial ergonovine stress tests on a typical positive animal (S-T segment depression) using IV at 25 mm./sec.

animals. Figures 4 and 5 illustrate typical occlusive atherosclerosis of a coronary artery and an area of extensive fatty metamorphosis respectively. An interaction between the nicotine and cholesterol is suggested, as none of the animals fed nicotine only (group 2) demonstrated either fatty metamorphosis or myocardial necrosis. Furthermore, there was no indication from the mortalities or any of the other studies that nicotine given alone produced malignant atherosclerosis.

Peripheral Vascular Changes. Changes in peripheral vascularity appeared in all groups receiving cholesterol. The effect was seen in the limbs as excessive scaling and reddening, followed progressively by gross swelling, alopecia localis, suppurative and sometimes bleeding lesions of all four paws and adjacent areas. These changes appeared first in the nicotine-cholesterol groups (4 to 6) between the 12 and 16 week periods, and progressed rapidly, while in the cholesterol only group (3) the lesions did not appear until after 22 to 23 weeks on the diet, and then progressed only slowly until the termination of the experiment. The fact that these findings were not seen with the nicotine only group (2) once again suggests an interaction between nicotine and cholesterol.

Several animals receiving cholesterol demonstrated a fatty infiltration of the eye. Not only were scleral deposits observed, as has been previously reported,[4] but the iris was also markedly infiltrated in some animals. There were no apparently significant differences between the cholesterol and nicotine-cholesterol groups in regard to the eye, and no attempt had been made to note differences in the times of onset.

DISCUSSION

From these studies it appears that an interaction occurred between nicotine and cholesterol in terms of mortality, electrocardiographic and pathologic evidence of coronary atherosclerosis, and peripheral vascular involvement. With one possible exception there was no apparent relationship between the effect of nicotine per se and any of the above criteria. This exception was the consistent production of thickening and fibrosis of the small coronary branches in the nicotine only group (3). Nevertheless, reinforcement of the

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FIG. 4 *Top.* Photomicrograph demonstrating the characteristic occlusive atherosclerosis of a coronary artery in a nicotine-cholesterol animal. Frozen section—Oil-red-O stain. Circa 60×.

FIG. 5 *Bottom.* Photomicrograph of a nicotine-cholesterol treated rabbit's heart showing details of extensive fatty metamorphosis bordering on infarction. Frozen section—Oil-red-O stain. Circa 120×.

observed cholesterol effects by nicotine were quite apparent. It is interesting that, although a 16 fold nicotine dose differential was employed, no dose-dependent effects were observed.

An earlier study with male rabbits and a 0.1 per cent cholesterol diet demonstrated that nicotine increased total serum cholesterol and phospholipid levels.[2] This was not confirmed by the present study on female rabbits and a 1 per cent cholesterol plus 5 per cent cottonseed oil diet. One explanation may be that the high cholesterol-oil diet literally flooded the system, thus masking the nicotine effect. It must also be considered that the two rabbit studies also differed in the sex of the animals as well as in the addition of cottonseed oil to the diet in the present study.

While it is not possible to define the mechanism of the interaction between nicotine and cholesterol from the experimental evidence, several points should be considered. As indicated above, the results cannot be attributed to differences in serum cholesterol or phospholipid levels. It may be significant that nicotine apparently did not affect the production of atheroma in the aorta, while evidence of increased atherosclerosis in the heart and periphery was quite apparent. Nicotine is known to exert a rather complex vascular effect in which vasoconstriction predominates. It is possible that prolonged constriction of the vessels involved or other undefined vascular actions of nicotine created the proper physical and/or metabolic environment for the deposition of cholesterol. The observation that the small coronary vessels were thickened and fibrotic in the nicotine only group (2) would strengthen this possibility.

SUMMARY

Groups of female albino rabbits were administered cholesterol, nicotine, and nicotine-cholesterol for a 24 week period. The addition of nicotine to the cholesterol regimen did not significantly affect body weight, serum cholesterol or lipid phosphorus or gross aortic atherosclerosis under the conditions of the test. The nicotine-cholesterol groups demon-

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strated greater mortality, as well as greater electrocardiographic and pathologic evidence of cardiac involvement and peripherall vascular changes, than did the cholesterol or nicotine groups. It is suggested that the combination of nicotine and cholesterol produces a cardiovascular interaction.

SUMMARIO IN INTERLINGUA

Gruppos de conilias albin recipeva administrationes de (1) cholesterol, (2) nicotina, e (3) nicotina e cholesterol durante periodos de 24 septimanas. Le addition de nicotina al regime de cholesterol non afficeva significativemente le peso corporee, le cholesterol del sero, le phosphoro lipidic del sero, o le apparentia macroscopic del atherosclerosis arterial. In le gruupo recipiente le duo agentes, le mortalitate esseva plus grande, e signos electrocardiographic e pathologic indicava plus evidentemente le presentia de affection cardiac e de alterationes periphero-vascular. Es exprimite le opinion que le combination de nicotina e cholesterol produce un interaction cardiovascular.

REFERENCES

1. MASLOVA, K. K.: Influence of nicotine on experimental atherosclerosis. Bull. Exper. Biol. Med. 41: (420-1-1432) 1956.
2. WENZEL, D. G., AND BECKLOFF, G. L.: The effect of nicotine on experimental hypercholesterolemia in the rabbit. J. Am. Pharm. Assoc., Sci. Ed., 47: 338, 1958.
3. JOHNSTON, L. M.: Tobacco smoking and nicotine. Lancet 243: 742, 1942.
4. RINZLER, S. H., TRAVELL, J., KARP, D., AND CHARLESON, D.: Detection of coronary atherosclerosis in the living rabbit by the ergonovine stress test. Am. J. Physiol. 184: 605, 1956.

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